

# Increase in the Systolic Pressure of Rats Chronically Fed Cadmium

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In our laboratory, chronically feeding cadmium to groups of rats has been reproducibly associated with average increases of 15 to 20 mm Hg in systolic pressure. A total of 497 female Long-Evans rats were continuously provided with drinking water fortified with five essential elements and containing from 0.01 to 50 ppm cadmium, as the acetate, from weaning for as long as 30 months. These rats, plus 311 matched control animals which received fortified water without added cadmium, were fed a special low-cadmium diet. All 808 rats were weighed at least monthly as a screen for cadmium toxicity, and their systolic pressures were measured every 3 or 6 months. The two lowest concentrations of cadmium tested (0.01 and 0.03 ppm) were not pressor; the three highest concentrations (10, 25, and 50 ppm) ultimately proved to be toxic.

All indirect systolic pressures (each measured in triplicate) of all rats which received 0.1 to 5 ppm cadmium (i.e., nontoxic pressor doses) averaged 15.0 mm Hg more than simultaneously measured pressures of control rats. This average increase over the control pressure is extremely significant statistically, even though it seems relatively small in absolute terms. Occasionally, however, some rats had much larger than average increases in pressure; thus, 10 of 60 rats receiving from 0.1 to 0.5 ppm cadmium for 18 months had systolic pressures that were more than 50 mm Hg above the average pressure of the control rats. Cadmium-induced hypertension is not limited to females or to a particular strain. Although we have usually used one strain of female Long-Evans rat from a single source, males of the same strain and female Sprague-Dawley rats have also developed comparable hypertension.

All subgroup II elements can apparently induce similar increases in systolic pressure averaging 15 to 20 mm Hg, but cadmium is pressor in much smaller amounts than mercury or zinc. Thus, to induce a demonstrable increase in pressure requires more than ten times as much divalent mercuric ion as cadmium and more than 1000 times as much zinc as cadmium. Exposure to another metal along with cadmium can markedly alter the ability of cadmium to induce hypertension. Selenium protects against the hypertension induced by twice as much cadmium. Large excesses of both zinc and copper have also inhibited the induction of hypertension by cadmium. In contrast, lead, which like cadmium, can also induce hypertension, augments rather than inhibits cadmium-induced hypertension; thus, lead and cadmium together can induce an *average* increase in systolic pressure in excess of 40 mm Hg, at least twice as large as is usually induced by either metal alone.

## Introduction

In 1962, Schroeder et al. first reported that chronic cadmium feeding could induce hypertension in rats; their report indicated that the induced hypertension was marked (1). They extended these observations in 1964 (2). In 1967, however, this group of investigators revised the magnitude of the induced hypertension sharply downward by considering the average change in pressure for the entire exposed rat population rather than just for those

animals which developed hypertension, i.e., the "responders" (3). Throughout, these investigators used standard conditions, including a standard dose of 5 ppm cadmium in drinking water (1).

My purpose here is to summarize the extensive observations which our group has made on the blood pressures of rats chronically fed cadmium. We were able to confirm that long-term cadmium ingestion can induce hypertension, but the magnitude of the effect was less than expected from Schroeder's reports. It therefore seemed prudent to repeat our observations to demonstrate their reproducibility before starting to investigate mechanisms. Thus, much of our work to date has been designed to demonstrate the pressor effect and

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to define its magnitude and the amount of exposure necessary to evoke it. Two general types of data are presented below. The first deals with the cadmium-induced effects on blood pressure and the second with the changes in these effects brought about by other metals.

We have studied eight populations of animals, involving a total of 808 Long-Evans female rats, which were handled under standard conditions and exposed to cadmium alone in one of 10 concentrations, ranging from 0.01 to 50 ppm, or used as litter-mate controls for such animals. The average systolic pressures cited were obtained from groups which initially contained at least 15 rats; in determining these averages all animals in the group were included. Six groups of rats totaling 144 animals were exposed to 5 ppm cadmium, duplicating Schroeder's standard conditions; the average cadmium-induced increases in systolic pressures for the groups ranged from 6 to 23 mm Hg.

Since the 5 ppm cadmium dose is considerably above the intake of the average adult American (on the basis of milligrams cadmium ingested per kilogram body weight), a range of cadmium exposures was tested. Essentially the same pressor effect was found with exposures ranging from 0.1 to 5 ppm cadmium. Exposures below 0.1 ppm had little pressor effect, and exposures above 5 ppm were eventually toxic. Although separate subpopulations of blood pressure "responders" and nonresponders could not be recognized in most of our rat populations, at least one unusually responsive subpopulation developed marked hypertension following exposures to less than 1 ppm cadmium. Having confirmed cadmium-induced hypertension in female Long-Evans rats, the effect was shown to occur in males and in another strain of rats; thus 5 ppm cadmium was pressor for male Long-Evans and female Sprague-Dawley rats.

\* Our initial cadmium-feeding studies in 1970 adhered as rigorously as practical to the standard experimental conditions established by Schroeder's group (1); we obtained our rats, and even our rye, from the same suppliers. We argued that failure to induce hypertension would not be definitive if the conditions were altered. However, because the plastic cages with stainless steel lids, which Schroeder used, placed severe constraints on the number of animals we could follow at any one time, our original experiment tested both the plastic cages with stainless steel lids and all stainless steel cages. The same degree of hypertension, i.e., difference between the pressures of the cadmium-exposed and control rats, was apparently induced in both types of cages, although the average systolic pressure of both the control and cadmium exposed populations were higher by almost 10 mm Hg in the stainless steel cages (4). In order to use large numbers of rats, we opted to work with the much more convenient stainless steel cages and accept the higher control pressures. Except for this single experiment where we tested the plastic cages, we have used the stainless steel cages throughout.

Because of the modest size of the average pressor effect associated with cadmium, conditions were sought which might increase its magnitude. It was observed that all subgroup II metals can induce similar hypertension, although cadmium is effective at much lower concentrations than either non-essential mercury or essential zinc. Moreover, when exhibited with cadmium, at least three metals inhibit the pressor response to cadmium: selenium, zinc, and copper. In contrast, at least one metal, lead, can significantly augment the pressor response to cadmium.

## Methods

Groups of at least 15 weanling female Long-Evans rats from Blue Spruce Farms in Altamont, New York, were treated as described by Schroeder (1) and modified slightly in our laboratory (4, 5).<sup>\*</sup> On arrival, the rats were placed in a low contamination environment; they were all fed the same rye-based low-cadmium diet *ad libitum* and given free access to fortified drinking water containing any metals which were to be exhibited. Like the diet, the drinking water was made as described by Schroeder. It consisted of deionized water with a resistance of 5 megohms "fortified" by the addition of cobalt (1 ppm), molybdenum (1 ppm), copper (5 ppm), manganese (10 ppm), and zinc (50 ppm) (1, 5). Random batches of the diet were assayed regularly and found to contain an average of  $0.0137 \pm 0.0019$  (SD)  $\mu\text{g}$  cadmium/g food. For the other trace metals that were assayed, the following average amounts (in  $\mu\text{g/g}$ ) were found: zinc, 35; mercury, < 0.005; selenium, 0.37; iron, 42; manganese, 23; copper, 3.3; nickel, 0.29; and chromium, < 0.05. In addition, the following average amounts (in mg/g) of other metals were present: sodium, 5.9; calcium, 3.8; potassium, 7.6; and magnesium, 1.4. Food intake throughout the life of the rat averaged approximately 20 g/rat/day.

The metals to be tested were administered in drinking water made by dissolving cadmium, zinc, or lead acetate, mercuric or cupric chloride, or sodium selenite in the "fortified" deionized water. (Studies with radioactive tracers indicated that there was no significant precipitation and that all metals remained in solution. The only exception to this was in some experiments with hard water, not considered here.) Although there was variability between both individual and group means, the average intake of water for a single rat approximated 15 ml daily at the start of the experiment and rose in a roughly linear fashion to about 25 ml by 12 months after which it remained relatively constant.

Systolic pressure was determined in triplicate in

lightly anesthetized (0.25 mg intraperitoneal pentobarbital/kg body weight) animals by the tail cuff method, as previously described (5). [Although only systolic pressure is reported here, consistently parallel changes in systolic and diastolic pressures have been regularly observed and previously reported (4). Direct determination of diastolic pressure requires cannulation of the artery which is a major procedure and results in sacrifice of the vessel. It cannot therefore be routinely used for serial blood pressure determinations. The directly determined systolic pressure averaged 6 mm Hg lower than the indirectly measured pressure.] During their entire stay in our laboratory, animals were removed from their cages for only two purposes: (a) determination of systolic pressure at intervals of three to six months and (b) weekly cage cleaning and group weighing. Measuring pressure was done at long intervals to ensure that the animals were uninfluenced by the anesthetic or its after effects for the very large majority of their lives. Weighing was used as an index of group well-being. Except as specifically indicated below, the average weights of the experimental groups did not differ significantly from those of the control animals.

## Results

### Standard 5 ppm Cadmium Exposure

We have measured the effect of the standard exposure to drinking water containing 5 ppm cadmium in six separate populations of female Long-Evans rats. The average cadmium-induced increases in

systolic pressure are shown at four different times in Figure 1. After six months of cadmium exposure, the average difference in systolic pressure between the cadmium-fed groups, which totalled 144 animals, and their 267 control animals was 12.4 mm Hg, with a standard deviation of 13.9 mm Hg. This difference was statistically significant with a  $p$  value of  $< 0.001$  ( $t = 8.88$ ). The average difference rose to 16.6 mm Hg after 12 months ( $t = 6.55$ ) and became maximal at 21.5 mm Hg after 18 months ( $t = 8.38$ ).

The six separate populations of rats exposed to 5 ppm cadmium all had similar pressor responses (Table 1). After six months, all six had mean increases in systolic pressure, which ranged from 6 to 20 mm Hg and averaged 12.4 mm Hg. After 12 months, the mean increases ranged from 9 to 22 mm Hg and averaged 16.6 mm Hg.

### Range of Cadmium Exposures

In Figure 2, the average cadmium-induced increase in systolic pressure is examined as functions of two aspects of exposure, its length and the dosage used. Increases with successively doubled exposure periods from 3 to 24 months and a hundred-fold range of cadmium concentrations from 0.1 to 10 ppm are presented. The lowest cadmium concentration and the shortest exposure induced only a small increase in pressure, averaging 5 mm Hg. The same concentration and doubled exposure induced an increase of 10 mm Hg. The remaining bars from left to right in the graph generally trended slightly upward from the 10 mm Hg increase toward an increase of 15 mm Hg.

Table 1. Increase in systolic pressure in six separate experiments after 6 and 12 months exposure to standard 5 ppm cadmium.<sup>a</sup>

Date Cd feeding began	6 months cadmium feeding					12 months cadmium feeding		
	Control		5 ppm Cd		Increase, mean, mm Hg	Control, mean $\pm$ SD, mm Hg		Increase, mean, mm Hg
	No. of rats	Mean $\pm$ SD, mm Hg	No. of rats	Mean $\pm$ SD (mm Hg)			5 ppm Cd, mean $\pm$ SD, mm Hg	
Oct. 21 '70 <sup>b</sup>	(64)	112 $\pm$ 15 <sup>c</sup>	(32)	125 $\pm$ 17	13	110 $\pm$ 14 <sup>c</sup>	127 $\pm$ 20	16
Aug. 7 '74	(60)	99 $\pm$ 13	(20)	112 $\pm$ 11	13	102 $\pm$ 9	111 $\pm$ 10	9
Mar. 26 '75	(40)	106 $\pm$ 8	(20)	112 $\pm$ 12	6	103 $\pm$ 13	119 $\pm$ 13	16
Aug. 18 '76	(43)	103 $\pm$ 12	(42)	115 $\pm$ 12	12	98 $\pm$ 18	117 $\pm$ 18	19
Dec. 1 '76	(15)	103 $\pm$ 12	(15)	113 $\pm$ 12	10	107 $\pm$ 9	129 $\pm$ 10	22
Sept. 1 '77	(45)	99 $\pm$ 11	(15)	119 $\pm$ 11	20	—	—	—
Total	(267)	104.1 $\pm$ 13.2	(144)	116.5 $\pm$ 13.9	12.4	103.3 $\pm$ 13.1	119.9 $\pm$ 16.8	16.6

<sup>a</sup> Average systolic pressures  $\pm$  standard deviations (SD) at 6 and 12 months are tabulated for all rats exposed to the standard 5 ppm cadmium under standard conditions. In addition, the numbers of rats included at 6 months are tabulated (in parenthesis); the numbers were essentially the same at 12 months. Finally, the average cadmium-induced increases in systolic pressure at 6 and 12 months are also tabulated.

<sup>b</sup> The October '70 experiment included a second population of control rats housed in plastic cages designed to decrease contamination, as described by Schroeder (1). These rats had an average control pressure of 103 mm Hg (4); they have not been included here because they were not handled in our standard manner. The small numbers (5 per group) of "Population II" rats from the October '70 experiment have not been included here; their pressures have been reported previously (5).

<sup>c</sup> The control pressures in our first experiment averaged almost 10 mm Hg higher than in subsequent experiments, possibly because our technique for excluding environmental cadmium was less well developed.

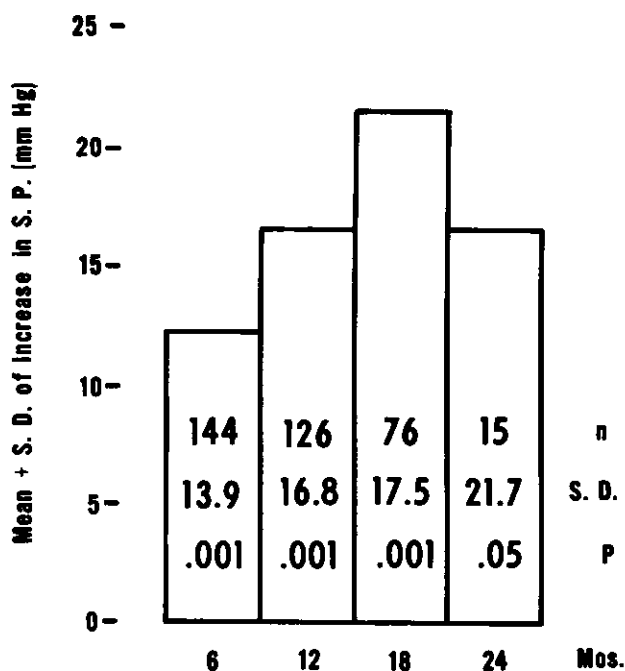


FIGURE 1. Pressor effect from varying exposure to standard 5 ppm cadmium. Average increases in systolic pressure of six rat populations exposed to 5 ppm cadmium for 6, 12, 18, and 24 months. The numbers of rats involved in each average, the standard deviation, and the statistical significance ( $p$  value) are indicated by the numbers in the columns. All increases have been corrected for any change in the average systolic pressure of litter-mate control rats handled similarly but without cadmium (see Table 1).

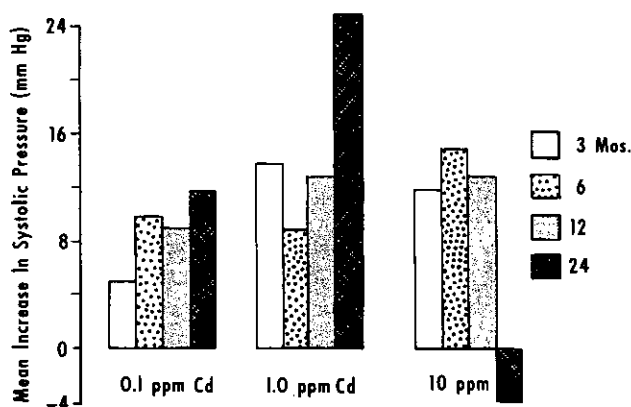


FIGURE 2. Pressor effect as function of length and intensity of cadmium exposure. Average changes in systolic pressure induced by three dosage levels of cadmium after successively doubled exposure intervals. Initially there were 15-60 rats in each cadmium-fed group, and 45-75 control rats against which increases in pressure were measured.

With groups of the size used here, there is some uncertainty regarding any specific difference cited in the graph. For example, although the effect of 0.1 ppm cadmium during the first year of exposure was somewhat smaller than the effect of 1.0 or 10 ppm, at subsequent times not shown on the graph, the increases were relatively large (i.e., at 15, 18, and 21 months, they averaged 16, 18, and 15 mm Hg, respectively). There is some confirmation for the unusually large increase of 25 mm Hg after 24 months exposure to 1 ppm cadmium, since 2.5 ppm cadmium induced a comparable increase of 24 mm Hg at 24 months. These large increases involved relatively small numbers of rats; the two groups included only 9 and 12 two year survivors, respectively. There was little uncertainty, however, about the decrease in pressure after 24 months of exposure to 10 ppm cadmium; this change was typical of a consistent pattern of toxicity following prolonged exposure to high concentrations. Incipient toxicity was already evident at 18 months when the induced change in pressure, although still positive, averaged only 7 mm Hg, as shown in Figure 3.

Table 2 summarizes our average systolic pressure data for groups of rats exposed to cadmium alone under our standard conditions. At one extreme, we never observed an average increase in pressure of as much as 10 mm Hg with exposures of 0.01 or 0.03 ppm cadmium, although the increase after 12 months was of borderline significance ( $p < 0.05$ ), raising the question of whether some rats may have a pressor response to even these low concentrations (6). At the other extreme, there was an early increase in pressure for the 50 ppm group ( $p < 0.025$  after 6 months exposure), but thereafter that exposure was depressor; this concentration was overtly toxic and markedly inhibited growth in weanlings from the very first. Similarly, 25 ppm cadmium induced an increase in pressure for a year ( $p < 0.005$  at 6 months and  $p < 0.025$  at 12 months), but it then became depressor; it too inhibited growth, albeit minimally. A level of 10 ppm cadmium was significantly pressor for a year, but from 18 to 30 months there was stepwise conversion of its pressor effect to a depressor effect; it never inhibited growth significantly. [Additional data for high cadmium exposures are available (5)].

For the intermediate concentrations from 0.1 to 5 ppm cadmium, the average increases in pressure ranged from 5 mm Hg for the former after three months of exposure to 33 mm Hg for the latter after 30 months of exposure. Three quarters of the 38 tabulated averages for this intermediate range of concentrations represent increases of 10 to 20 mm Hg, suggesting that this is the usual response to a wide range of cadmium exposures under our stan-

dard conditions. From the data for 18 months of exposure, the relatively constant average increase of 15 to 20 mm Hg to a range of cadmium concentrations is well shown in Figure 3.

### Subpopulation of Cadmium Responders

Although the distribution pattern of individual pressures following cadmium exposure was difficult

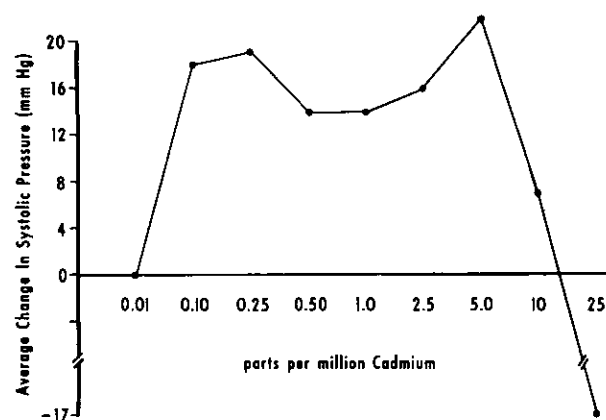


FIGURE 3. Exposure to average changes in systolic pressure induced by nine exposure levels of cadmium (0.01 to 25 ppm Cd) for 18 months. At this time, there were still at least 13 rats in all groups, and the groups exposed to 0.1, 5, and 10 ppm Cd each contained more than 30 rats.

to define precisely because of relatively small sample size, distribution usually appeared to be relatively normal (4). Occasionally, however, there seemed to be a subpopulation of responders in which cadmium induced marked hypertension. This phenomenon was most obvious for our August 7, 1974, experiment involving 140 rats. After 18 months, one-sixth of the rats exposed to 0.1, 0.25, and 0.5 ppm cadmium had systolic pressures which exceeded the control mean by four standard deviations, i.e., by 50 mm Hg. In contrast, none of the litter-mate controls or 5 ppm cadmium rats had this degree of hypertension (Table 3) (7).

### Pressor Response of Male and Sprague-Dawley Rats to Cadmium

Since our early work was limited to female Long-Evans rats, it seemed important to demonstrate that the effect was not limited to one sex or one strain of rats. We, therefore, tested the standard 5 ppm cadmium in male Long-Evans rats and in female Sprague-Dawley rats. After a year of exposure, cadmium had induced a typical increase of 17 mm Hg in the males, although both control and cadmium-exposed males had higher pressures than females (8). The data for the female Sprague-Dawley rats were more limited because the colony

Table 2. Mean increase in systolic pressure after varying cadmium exposure.<sup>a</sup>

Months of Cd feeding	No. of control rats tested	No. of Cd fed rats (total)	Increase in systolic pressure at various levels of cadmium in drinking water from time of weaning, mm Hg <sup>b</sup>										
			0.01 ppm	0.03 ppm	0.10 ppm	0.25 ppm	0.50 ppm	1.0 ppm	2.5 ppm	5.0 ppm	10 ppm	25 ppm	50 ppm
3	175	165			5			14	6	12	12		
6	311	463	-1	0	10**	22	11	9**	10**	12**	15**	12	11
9	58	56								16**			
12	238	341	9	8	9*	12	11	13*	15	17**	13*	9	-13
15	166	211	2	3	12*	12	10	17		12*			
18	191	263	0	1	18*	19	14	(14)	16	22**	7*	-17	(-12)
21	32	30			15					(11)			
24	85	118			(12) <sup>b</sup>	(7)	(15)	(25) <sup>b</sup>	(24)	17	-4	(-20)	(-27)
30	21	39						(17) <sup>b</sup>	(33) <sup>b</sup>	(-12) <sup>b</sup>	(-14) <sup>c</sup>		(-30) <sup>b</sup>
Totals <sup>c</sup>	311	497	20	20	55	20	20	66	44	144	76	16	16
Means <sup>d</sup>	105.0		105.7	106.3	113.1	115.5	112.6	117.5	125.1	120.0	117.8	113.6	108.1

<sup>a</sup> Average increases in systolic pressure of cadmium-fed rats (corrected for any change in average control pressure) are tabulated for totals of 311 control rats and 497 cadmium-fed rats. Eight separate experiments with eight different populations of rats are included (one begun in May and another in September of 1973, which involved no rats exposed to 5 ppm cadmium, plus the six listed in Table 1). This comprises all of our systolic pressure data on groups of 10 or more rats fed cadmium alone under standard conditions.

<sup>b</sup> All entries which include data from more than 30 rats are marked with one asterisk, and those with data from more than 50 rats are marked with two asterisks. No group contained less than 15 rats at the outset of the experiment; any subsequent averages which included data from fewer than 15 rats have been placed in parentheses; and a superscript indicates the actual number when fewer than 10 rats were included. The standard deviations of the tabulated averages ranged from 9 to 39 mm Hg, with 9/10 of them being less than 20 mm Hg and 3/4 less than 15 mm Hg. Using Student's *t* test, a change of 8-9 mm Hg usually had *p* < 0.05, and one of 12 or more mm Hg had *p* < 0.001 mm Hg.

<sup>c</sup> The totals row of the table indicates the number of rats which began a given exposure. The values in columns 2 and 3 are not obtained by summing the nine entries in the column, since each rat had its blood pressure measured more than once.

<sup>d</sup> Means were obtained by averaging each pressure obtained from each rat. The values are lowered by the relatively low values after short exposures, and they are also moderated by the decreasing number of rats which survived long exposures.

developed a respiratory infection after nine months of cadmium exposure, but at six months there was a significant pressor effect in the cadmium-fed animals as compared to the controls (8).

## Similar Pressor Responses to All Subgroup II Metals

Chronic feeding of an appropriate concentration of any subgroup II metal is pressor in female Long-Evans rats. Although our data on zinc and mercury are not extensive and therefore have considerable uncertainty, all three subgroup II metals appear to induce average increases of 15 to 20 mm Hg in the systolic pressure, but the inducing doses differed by more than a thousand-fold (Table 4). Moreover, there is no pressor response when the challenging dose is increased to overtly toxic doses of nonessential cadmium or mercury.

The pressor response to chronically ingested cadmium has been described above. Exposure to 5 or 10 ppm mercuric ion for 12 months was pressor, whereas neither 2.5 nor 25 ppm was pressor, with the latter concentration being toxic (4). The situation was more complicated in the case of zinc which is essential and present to the extent of 35 ppm in our standard diet and to the extent of 50 ppm in the "fortified" water eaten and drunk by all of our rats.

No effect on pressure was observed if the zinc in the water was decreased from 50 ppm to zero; however, if it was increased from 50 to 75 ppm, the typical pressor response to cadmium and mercury was observed (9). Both 100 and 200 ppm zinc were pressor, and no search was made for a larger toxic but non-pressor concentration of zinc.

## Inhibition of Cadmium Effect by a Second Metal

A second metal can either inhibit or enhance cadmium-induced hypertension. The three essential metals: selenium, zinc, and copper have each been shown to inhibit the pressor effect of cadmium; inhibition has ranged from 60 to 100%. Table 5 presents our copper data and the data for one of three similar experiments involving selenium and zinc. The inhibition studies were among our early experiments and were carried out while we were still using relatively large cadmium exposures. In brief, the pressor effects of both 2.5 and 10 ppm cadmium were inhibited by half as much (i.e., by half the molarity of) selenium (10). The pressor effects of 2.5 and 10 ppm cadmium were also inhibited by 100 and 200 ppm (i.e., by 70 and 35 times the molarity of) zinc. The pressor effect of 5 ppm cadmium was also inhibited by 20 ppm (i.e., by 8 times the molarity of)

Table 3. Distribution of systolic pressure (SP) for one rat population.<sup>a</sup>

Cadmium dose, ppm	Mean SP $\pm$ SD after 18 months, mm Hg	Rats with SP more than 2 SD above control mean, % > 125 mm Hg	Rats with SP more than 4 SD above control mean, % > 150 mm Hg
0	100.5 $\pm$ 12.4	4	0
0.1	124.3 $\pm$ 15.1	33	17
0.25	119.6 $\pm$ 19.8	44	19
0.5	114.5 $\pm$ 16.9	20	13
5.0	114.0 $\pm$ 15.0	13	0

<sup>a</sup> There were initially 60 control rats and 20 rats per experimental group. One-sixth of the rats exposed to 0.1, 0.25, and 0.5 ppm cadmium for 18 months had systolic pressures which exceeded the mean pressure of the control rats by more than four standard deviations (SD). No control rat and no rat exposed to the standard 5 ppm cadmium had this great a pressor response. The data presented here constitute a single complete experiment, i.e., a single complete population.

Table 4. Pressor effects of chronically feeding subgroup II metals.

Ion	Minimum toxic dose, $\mu M^a$	Minimum pressor dose, $\mu M^a$	Number of rats tested	Response to minimum pressor dose		Mean SP $\pm$ SD increase, mm Hg	Reference
				Dose, ppm <sup>a</sup>	Length, mos		
Zn <sup>2+</sup>	—	1100	14	75	15	20 $\pm$ 18	(8)
Cd <sup>2+</sup>	89	0.89	55	0.1	6	10 $\pm$ 11	This report
Hg <sup>2+</sup>	125	25.0	16	5	12	16 $\pm$ 12	(4)

<sup>a</sup> Minimum pressor and toxic metal concentrations in drinking water are presented in micromoles per liter to allow comparison between metals while the doses in cited experiments are given in parts per million to conform with the rest of the manuscript. Thus the concentrations of the third and fifth columns are the same. Toxic doses were not pressor.

Table 5. Inhibition of cadmium-induced hypertension by a second metal.<sup>a</sup>

Inhibitor exposure level, ppm	Cadmium exposure level, ppm	Pressor effect of inhibitor alone, % of Cd effect		Inhibitory action of inhibitor + cadmium, % decrease in Cd effect	
		6 Mos	12 Mos	6 Mos	12 Mos
Se (0.9)	(2.5)	133	40	Complete	93
Se (3.5)	(10)	39	35	72 <sup>b</sup>	Complete <sup>b</sup>
Zn (100) <sup>c</sup>	(2.5)	None	167	Complete <sup>b</sup>	60
Zn (200) <sup>c</sup>	(10)	56 <sup>b</sup>	80 <sup>b</sup>	67 <sup>b</sup>	60 <sup>b</sup>
Cu (20) <sup>c</sup>	(50)	None	32	92 <sup>b</sup>	89 <sup>b</sup>

<sup>a</sup> The concentrations of inhibiting metals and of cadmium are presented in the first two columns, and the pressor effects of the inhibiting metals alone and their inhibition of the cadmium pressor effects are presented in the next four columns. Four groups of rats provide the data for each of the five rows of figures in the table; there was a group with neither cadmium nor inhibitor, a group with cadmium alone, a group with inhibitor alone, and a group with both cadmium and inhibitor.

<sup>b</sup> These effects were statistically significant with  $p < 0.01$ . The size of the groups of animals receiving 0.9 ppm selenium and 100 ppm zinc were small, and these data are included primarily to indicate trends.

<sup>c</sup> Since the "fortified" drinking water contained 50 ppm zinc and 5 ppm copper, the zinc concentrations used here represented excesses of 50 and 150 ppm zinc above that given to the control rats, and the copper concentration represented an excess of 15 ppm copper (8).

copper. As indicated previously, zinc itself can be pressor; moreover, a possible, but not statistically significant, pressor effect has also been observed for selenium, but not for copper (Table 5).

### Augmentation of Cadmium Effect by Lead

Unlike the metals mentioned above, lead augmented the pressor effect of cadmium. By itself lead produced a pressor effect comparable to that produced by cadmium. In two separate experiments, when lead and cadmium were administered together, their effects were at least additive. The first experiment involved only one concentration of each metal but has now continued for 18 months; the second involved three concentrations but began only six months ago. Figure 4 presents the largest average increase in systolic pressure we have yet observed. This increase occurred after three months exposure to 1 ppm of both metals; it averaged 43 mm Hg above the control mean and was more than three times the average increase with either cadmium or lead alone. In the same experiment, the additive pressor effect was also seen with a smaller concentration (0.1 ppm) after a longer exposure (6 months). Thus, the average systolic pressure for 45 control rats was 99 mm Hg. The average increase in the systolic pressure for 15 rats exposed to 0.1 ppm cadmium for six months was 13 mm Hg. The average increase for 15 rats exposed to 0.1 ppm lead was 10 mm Hg, and the average increase for 15 rats exposed to 0.1 ppm of both cadmium and lead was 27 mm Hg (11).

## Discussion

### Description of Findings By Others and Possible Explanation of Seeming Discrepancies

The data presented here indicate that, with slight modifications of Schroeder's standard conditions,

we were able to confirm his observations that long-term, low-dose cadmium feeding can induce hypertension. There are some other published confirmatory data, but they are sparse. Sorenson et al. have reported a pressor effect in male rats following chronic cadmium feeding (12), and Ohanian has reported a cadmium-induced pressor response in "Dahl hypertension-sensitive" rats (13). These few confirmatory observations must be coupled with equally sparse reports of failure to induce hypertension with cadmium feeding (14-16).

The paucity of positive reports could be related in part to the long lag period inherent in such experiments, and there may be various partial explanations for the several reported failures to induce hypertension by chronic cadmium feeding. Schroeder made the original observation in 1962 (1) and was able to repeat it many times (2, 3, 17). Our initial confirmation by abstract was in 1971 (18); our preliminary paper was in 1974 (4), and a complete report was not made until 1977 (5). In attempting to confirm Schroeder's observations, we opted to adhere as closely to his conditions as possible, obtaining our rats and initially even our rye from his suppliers, feeling that we would have to repeat his experiment with no deviations, if we altered his conditions and failed to induce hypertension. Since adopting his conditions, we have observed cadmium-induced hypertension in each of eight experiments carried out in our laboratory. Minor changes in the experimental conditions, which have been consistently used in both Schroeder's laboratory and in our laboratory, may well be very important. We have already found it possible both to inhibit the effect completely and to augment it markedly. Perhaps the rye-based diet is particularly well suited for such experiments since Schroeder's group and our group have both used it exclusively as the major dietary ingredient.

Because of the large range of cadmium exposures which can produce a near maximal pressor response, minimal unrecognized exposure could lead to hypertension in the control group which would then mask any hypertension in the experimental group. As little as 0.1 ppm cadmium in drinking water, amounting to an intake of about 2  $\mu\text{g}$  of cadmium per day, induces hypertension, and there is frequent failure to realize the potential for unrecognized cadmium exposure in the usual animal facility. Likewise there has been frequent failure to realize that toxicity, which seems to be incompatible with hypertension, may be induced by what is often considered as moderate cadmium exposure. Facilities which can minimize extraneous cadmium exposure and still maintain healthy animals for years are rare, yet both conditions seem to be important. Anything which renders the animals overtly sick or even prevents them from gaining weight and growing in an optimum manner seems liable to inhibit cadmium-induced hypertension. Intercurrent pneumonia proved to be by far the most common such problem in our rat colonies during the 1960's.

Cadmium-induced hypertension may also have been overlooked because it was not as marked as indicated in Schroeder's original descriptions (1, 2).

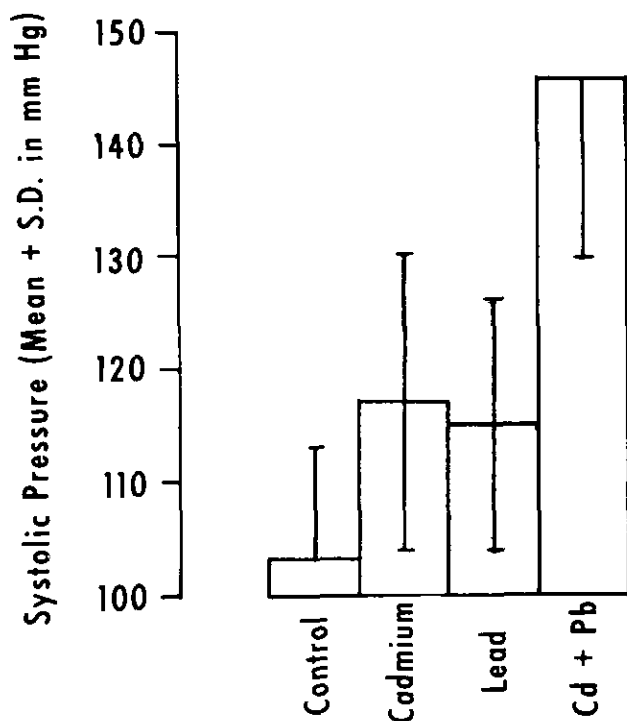


FIGURE 4. Augmentation of cadmium-induced hypertension by lead. Average systolic pressures  $\pm$  standard deviations are presented for 45 control rats and for groups of 15 rats fed 1 ppm cadmium and/or lead for three months.

There is frequent failure to realize that his subsequent reports indicated much lesser degrees of cadmium-induced hypertension (3) and that the large average increase in pressure which was initially reported was limited to a group of "responders." As indicated in Table 3, we have observed subpopulations of responders (7), but they are the exception rather than the rule.

### Possible Mechanisms and Significance of Cadmium-Induced Hypertension

The mechanism of the hypertension induced in rats by chronically feeding them low doses of cadmium has not been defined, although the following effects of injected cadmium suggest several possible mechanisms: (a) increased sodium retention (15, (19); (b) direct vasoconstriction (20); (c) hyperreninemia (21); and (d) increased cardiac output (20). Although hyperreninemia has been observed following fed as well as injected cadmium (21), the acute hypertensive effect of injected cadmium is not dependent on renin (22). On the other hand, although increased cardiac output has only been observed immediately after intraarterially injected cadmium, the standard 5 ppm dose of cadmium can produce significant cardiac effects; thus, Kopp, working with animals exposed to 5 ppm cadmium for 18 months in our laboratory, has observed depressed myocardial excitability, decreased high energy phosphate in the heart, and morphologic changes in cardiac muscle (23). Finally, without elucidating mechanism, "hypertension-sensitive Dahl rats" show a greater pressor response to intraarterial cadmium than do "hypertension-resistant Dahl rats" (24).

There is considerable variability in human exposure to environmental cadmium, and many of the details involving accumulation of the metals in man remain to be worked out. In the United States, the average cadmium intake is estimated to be 50  $\mu\text{g}$ /day or just below 1  $\mu\text{g}$ /kg-day (25). After 40 or 50 years, this results in an average renal cadmium concentration of 30  $\mu\text{g}$ /g of kidney or 3 mg/g of renal ash (26). Rats ingesting the lowest pressor dose of cadmium (0.1 ppm) have an intake of about 5  $\mu\text{g}$ /kg-day; after 18 months of this intake, their kidneys contain 1  $\mu\text{g}$ /g (6). Thus, they ingest about five times as much cadmium (per unit body weight) as the average American, but their renal cadmium concentration is only one-thirtieth as high. Rats exposed to 5 ppm have renal cadmium concentrations of about 40  $\mu\text{g}$ /g (5), which is similar to the average for adults in this country.

Cadmium-induced hypertension in rats, although reproducible, is usually relatively small in mag-



nitide and thus frequently considered of little consequence; however, most human hypertension is mild. Of the 60 million Americans who are now estimated to have hypertension (27), more than 80% have diastolic pressures which are usually below 105 mm Hg. Nonetheless, in man at least, even such mild elevations in blood pressure double the risk of heart attack and stroke, the major cardiovascular causes of disability and death (28). Moreover, like cadmium-induced hypertension in rats, mild essential hypertension in man has no obvious associated findings. In rats, the small amounts of cadmium that induce hypertension produce none of the usual toxic manifestations of more extensive exposure to cadmium, while, in man, the absence of symptoms is a major part of the problem in identifying mild hypertension and convincing the involved individuals of its ominous prognostic significance.

It must be emphasized that heavy, and certainly toxic, cadmium exposure is not associated with hypertension in rat or man. In rats 0.1 to 5 ppm cadmium induces hypertension but 10 to 50 ppm cadmium does not or only does so during the early portion of the exposure before toxicity becomes evident. In man, itai-itai disease and chronic cadmium poisoning are not characterized by hypertension (29); moreover, individuals with malignant hypertension and shrunken kidneys, consisting almost entirely of scar tissue with little parenchyma, have no excess renal cadmium (30). If cadmium plays any part in human hypertension, it is early in the course of the disease. By the time severe hypertension and compromised renal function have occurred, most of the accumulated renal cadmium has presumably been excreted in the urine in association with urinary protein.

Although the evidence that feeding cadmium can induce hypertension in rats seems convincing, there is no comparable evidence that cadmium is responsible for any part of human essential hypertension. The cause of essential hypertension remains unknown, however. For the moment, it can only be said that cadmium is a ubiquitous environmental contaminant which is frequently present in concentrations which can induce hypertension in rats and which, in animals at least, can have other cardiovascular effects (23). For the present it would certainly seem prudent to minimize cadmium exposure as much as economically practical while seeking answers as to whether cadmium has any effect on human blood pressure, or whether it produces any other undesirable effects.

## Conclusions

Under the conditions originally reported by Schroeder and slightly modified in our laboratory,

chronic ingestion of low doses of cadmium by rats reproducibly induces an increase in systolic pressure averaging 15 to 20 mm Hg. Occasionally the response is considerably larger, and the simultaneous exhibition of other metals can markedly influence it, with lead having the potential to at least double it. The extent, if any, to which these observations can be extrapolated from rat to man remains to be demonstrated. It is tempting to speculate whether these currently largely descriptive findings can be related to epidemiologic data of the type which associate cardiovascular mortality in man to the hardness of his water supply (31). The subject of the possible relationship between environmental cadmium and human hypertension certainly warrants further investigation.

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